

Self-Pack Column Options

for the CombiFlash[®] Rf System

Chromatography Application Note

AN23

Abstract

Occasionally, purification of compounds may be accomplished with media that are unable to be automatically packed into columns. An example is LH-20 and similar gels that swell upon exposure to solvent. The amount of swelling is solvent-dependent. Sometimes the purification is accomplished with a media that is not common, such as silver nitrate impregnated silica used to purify alkenes. The ability to pack columns allows one to take full advantage of the detection and safety features of the CombiFlash Rf system.

Anthocyanins from customer samples are run in LH-20 in a self-pack column and a glass column and with column adapter.

Self-pack Columns

Self-pack columns use the easy-to-use empty RediSep solid load cartridges and adjustable solid load cartridge caps. Adapters at the inlet and outlet allow the assembled self-pack column to be inserted between the column fittings on CombiFlash Rf system.

Example

A 25 g Self-pack Column Kit (PN 60-3877-207) was used for this experiment. LH-20 was swollen with methanol. The swollen LH-20 was transferred to a 25 g solid load cartridge to make a 10 cm bed. The top of the LH-20 was held in place with an upper frit from the kit. The



Figure 1: 25 g self-pack column installed on a CombiFlash Rf system

solid load cartridge cap was adjusted to reduce dead volume above the resin bed and the column was loaded on a CombiFlash Rf-200 system with visible wavelength detector (PN 68-523-006). The detection wavelength was 210 nm; flow rate was 10 mL/min. The column was run isocratically with 100% methanol. A sample load of 65 mg sample was injected. The method was modified to turn off the air purge (duration set to 0) to allow reuse of the column.

Advantages

The advantages of the self-pack columns include:

- Adjustable to match the bed height to the available sample mass
- Frits easier to use compared to sea sand commonly used to protect column bed
- No washing needed—discard the entire cartridge
- Column can be stored with end caps in place (do not store in hexane)
- Empty columns are inexpensive
- Multiple sizes available
- Load with whatever media is needed, such as CHP-20, LH-20, size exclusion gels, and others.

Disadvantage

- Limited to 50 psi

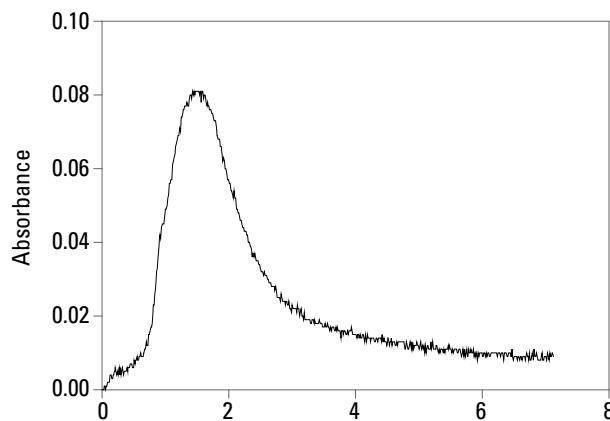


Figure 2: Chromatogram of self-pack column run

External Column Adapter

Example

A different anthocyanin sample from the Self-pack Columns example was used for this experiment. A glass column with threaded end fittings (30 cm length) was used for this experiment. A column adapter (PN 60-5394-432) as used to attach the column to the CombiFlash Rf system. The $\frac{1}{4}$ -28 fittings on the column adapter sufficed to connect the tubing to the adapter. The LH-20 was swollen in 1:1 methanol:water and loaded into the glass column. The column was run at 5 mL/min; a sample load of 100 mg sample was injected. The column was run with 100% methanol. The air purge was turned off to allow reuse of the column.

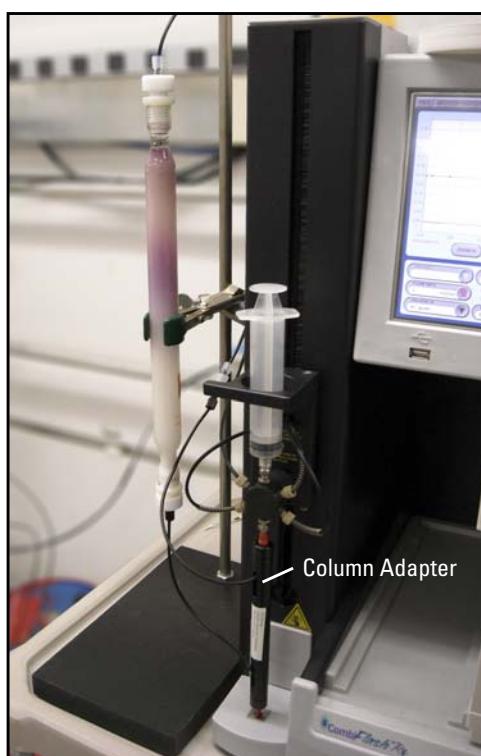


Figure 3: Glass column run on a CombiFlash Rf system using a column adapter

Conclusion

Customers have two options allowing them to use any chromatography media desired on a CombiFlash Rf system. Although these runs only needed isocratic elution, a CombiFlash Rf system could be used to run a gradient. Solvent mixing was done with the CombiFlash Rf system for the isocratic runs used here, saving time. Waste is avoided since the solvent was only mixed as needed and the remaining pure solvents could be used for other projects. The detector and fraction collector of the CombiFlash Rf system was used for both column options.

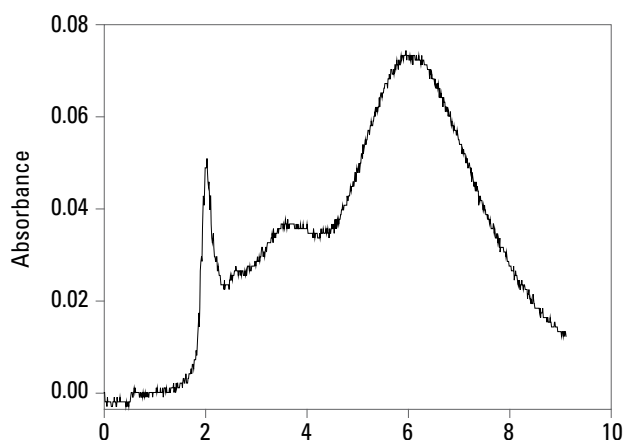


Figure 4: Chromatogram of glass column run

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